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Culture of vascular smooth muscle cells from small arteries of the rat kidney

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Culture of vascular smooth muscle cells from small arteries of the rat kidney.

Background. In contrast to arterioles, small arteries appear to be the preferential site of renal vascular smooth muscle cell (VSMC) proliferation under pathophysiological conditions. To date, techniques have been described to isolate renal arterioles and to culture VSMCs. The aim of the present study was to develop a method of culturing VSMCs from isolated small arteries of the rat kidney and to characterize their growth as compared with that of aortic VSMCs.

Methods. Renal vascular trees were isolated from kidneys of male Wistar rats by a sieving technique. VSMCs were grown from explants of collagenase-treated renal vascular trees and thoracic aorta. Growth curves and proliferation of renal and aortic VSMCs in response to fetal bovine serum (FBS) were compared by determination of cell number and DNA synthesis, measured as incorporation of 5-bromo-2′-deoxyuridine.

Results. Renal vascular trees consisted mainly of small arteries with a diameter of 80 to 400 μm (interlobar and arcuate arteries). As compared with total kidney or renal cortex, alkaline phosphatase activity was decreased by 81%, and vasopressin (10 μmol/L) was unable to stimulate adenylyl cyclase in renal vascular trees, indicating little tubular contamination. A homogenous population of spindle-shaped cells was cultured from renal vascular trees, which grew in a hill-and-valley pattern and stained positively for smooth muscle α-actin, according to the characteristics of VSMC phenotype. Renal VSMCs proliferated more slowly than aortic VSMCs and reached the plateau of growth at about half of the cell density of aortic VSMCs. Furthermore, proliferation of renal VSMCs depended more heavily on FBS concentration, since about threefold higher concentrations of FBS were needed for renal VSMCs to multiply at the same rate and to similarly stimulate DNA synthesis as compared with aortic VSMCs.

Conclusions. We present a method to culture renal VSMCs from small arteries of the rat kidney, which possess distinct growth characteristics as compared with aortic VSMCs.

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The vascular smooth muscle cell (VSMC) is the effector cell for the regulation of vessel tone. Renal vascular tone and hence hemodynamics are primarily controlled by the immediate action of a variety of vasoactive hormones [1]. It is well established that arterioles as well as small arteries participate in the regulation of hemodynamics in various nonrenal vascular beds [2, 3]. In the kidney, the contribution of small arteries to preglomerular vascular resistance is unknown. However, several studies suggest that small arteries may reduce renal blood flow in response to inflammatory mediators [4–6].

Long-term regulation of vascular tone involves growth processes (cell hyperplasia, cell hypertrophy, and remodeling) in the vessel wall, in order to adapt contractile force to vessel wall tension [7]. Furthermore, VSMC growth in the renal vasculature is altered under pathophysiological conditions. In contrast to afferent arterioles, small arteries of the kidney appear to be preferentially prone to deranged VSMC proliferation. In this regard, it has been shown in spontaneously hypertensive rats that wall hypertrophy occurs in small renal arteries, that is, interlobar and arcuate arteries, independently of systemic pressure [8, 9], whereas afferent arterioles remain unaffected [9, 10]. Furthermore, arthersclerotic lesions in experimental hypertension and proliferation of VSMCs in chronic renal transplant rejection appear to be confined to small arteries in the kidney [11–13].

Basically, two methods for culturing renal VSMCs have been described to date [14, 15]. In both methods, renal VSMCs are grown from preglomerular arterioles; small arteries are excluded by virtue of the technique used for vessel isolation. VSMCs of renal arterioles have been employed mainly to investigate renal VSMC signal transduction [15–20]. On the other hand, VSMC proliferation has been studied almost exclusively in cells cultured from the aorta, and findings have been extrapolated to VSMCs of other vascular beds, including the kidney. The aim of our study was therefore to develop a method for culturing VSMCs from small arteries of the rat kid-

ney, which appear to be the preferential site of pathological growth processes, and to compare growth characteristics of renal VSMCs with those of aortic VSMCs.

METHODS

Isolation of renal vascular trees

Male Wistar rats of about 250 g body wt (Iffa-Credo, l'Arbresle, France) were anesthetized with ether and were decapitated. Kidneys and thoracic aorta were removed, rinsed with sterile ice-cold phosphate-buffered saline (PBS; 1.5 mmol/L KH_2PO_4, 2.7 mmol/L Na_2HPO_4, 153.8 mmol/L NaCl), and placed in PBS on ice. If renal vascular trees were isolated for cell culture, the following steps were carried out under a laminar flow hood obeying sterile conditions. After complete and deep resection of the renal artery, kidneys were decapsulated and longitudinally bisected, and the medullae were removed. Kidney halves were pressed with their surface against stainless steel grids (Sigma, St. Quentin Fallavier, France) of 40 and 50 mesh size (420 and 300 μm openings, respectively). Renal vascular trees were gently rubbed against the grids and washed with cold isotonic saline. The optimal force, which was exerted on the vessels, represented a compromise between purity and viability, corresponding to high and low force, respectively. By this procedure, renal parenchyma passed through the meshes, while entire renal vascular trees were retained on the grid and could be picked up with fine forceps. Finally, renal vascular trees were extensively washed on a grid of 150 mesh size (100 μm openings) with a jet stream of cold isotonic saline.

Alkaline phosphatase activity

Alkaline phosphatase activity was measured in freshly isolated renal vascular trees, as well as in total kidney, at pH 10.5, using *p*-nitrophenylphosphate (*p*NPP) as substrate according to the colorimetric method described previously by Walter and Schütt [21]. Briefly, microvessels were suspended in saline and homogenized for three minutes in a Teflon-glass tissue grinder. Fifty $\mu g/mL^{-1}$ tissue protein were incubated in cold glycine-NaOH buffer, 50 mmol/L, pH 10.5, containing 0.5 mmol/L $MgCl_2$ and 5 mmol/L *p*NPP for 30 minutes at 37°C. The reaction was stopped by the addition of 0.4 mol/L NaOH, and the rate of formation of yellow *p*-nitrophenol was measured spectrophotometrically at 405 nm. Alkaline phosphatase activity was expressed as nmol *p*NPP hydrolyzed per minute and per milligram of protein.

Adenylyl cyclase assays

Basal, arginin-vasopressin (AVP; 1 and 10 μmol/L; Sigma)-stimulated and forskolin (10 μmol/L; Sigma)-stimulated adenylyl cyclase activities were measured in membrane fractions prepared from renal vascular trees, as well as from renal cortex. Isolated vascular trees were incubated for 20 minutes at 37°C in PBS containing 0.6 mg/mL collagenase from *Clostridium histolyticum* (type IA; Sigma) and 1 mg/mL soybean trypsin inhibitor (Sigma) in a shaking water bath ($100/min^{-1}$). After collagenase digestion, renal vascular trees were recovered and rinsed on a grid of 60 mesh size (250 μm openings) and cleaned on a grid of 150 mesh size with a jet stream of cold isotonic saline. Renal vessels were suspended in 5 mmol/L Tris-HCl buffer, pH 7.4, containing 0.25 mol/L sucrose, 1 mmol/L ethylenediaminetetraacetic acid (EDTA), 2 mg/L aprotinin, 1 mg/L leupeptin, and 1 mg/L pepstatin (Sigma) homogenized for three minutes in a Teflon-glass tissue grinder and centrifuged at $1000 \times g$ during four minutes (J6B Beckman). The pellet was resuspended in buffer and recentrifuged under the same conditions. Renal vascular membranes were prepared by pelleting the pooled $1000 \times g$ supernatants at $435{,}680 \times g$ during 10 minutes in a microultracentrifuge (TL100 Beckman) using a TLA 100.2 fixed-angle rotor. Renal cortical membranes were isolated from whole kidney by differential centrifugation using the method initially described by Fitzpatrick et al [22] and modified by Reynolds et al [23]. It has been well established that this membrane preparation consists mainly of basolateral tubular membranes free of brush border contamination [23]. Vascular and cortical membranes (8 μg) were incubated for adenylyl cyclase measurement, exactly as described previously [24]. Adenylyl cyclase activity was expressed as pmol cAMP formed per minute and per milligram of membrane protein.

Culture of renal VSMCs

Renal vascular trees were treated with collagenase as described previously in this article and transferred into 25 cm^2 culture flasks, which contained 1 mL Dulbecco's modified Eagle medium (DMEM; Life Technologies, Clergy Pontoise, France) supplemented with 20% fetal bovine serum (FBS; Bioproducts, Gagny, France), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). Culture flasks were coated with collagen type I from rat tail (Sigma) to prevent floating of explants. Culture flasks were incubated at 37°C in a humidified atmosphere of 10% CO_2 in air. Medium volume was increased as soon as the explants were firmly attached. Explants were removed from the culture flasks about 10 to 14 days later, when a sufficient amount of cells had grown out. After two to three weeks, cells were subcultured by trypsinization. Cells were passaged as they became confluent and were diluted 1:5. After the first subculture, cells were grown in antibiotic-free medium, consisting of DMEM supplemented with 20% FBS. The medium was exchanged every two days.

Culture of aortic VSMCs

Aortic VSMCs were obtained by the explant technique, as described by Chamley-Campbell, Campbell,

and Ross [25]. The thoracic aorta was thoroughly dissected free from connective tissue and cut open longitudinally. Intimal and adventitial layers were scraped with a scalpel blade, and the aorta was cut into small pieces. Aortic explants were processed, and aortic VSMCs were cultured in the same manner as it is described for renal VSMCs, with the exception that a concentration of 10% FBS was sufficient to sustain cell growth. The smooth muscle phenotype of cell lines was verified by positive immunofluorescence for smooth muscle α-actin.

Immunofluorescence

For antibody staining, cells were grown in chambers on glass slides (Life Technologies). Cells were rinsed with PBS, fixed with an ice-cold mixture of acetone/methanol (1:1) for 15 minutes and were dried in air. Dried slides were incubated in blocking solution (0.2% bovine serum albumin in PBS) for 20 minutes at room temperature. A FITC-conjugated monoclonal mouse anti-smooth muscle α-actin antibody (clone 1A4; Sigma) was added in a 1:100 dilution for two to three hours in a dark room. Slides were washed two times with PBS, covered with a cover slip, and sealed with nail polish. Slides were viewed under a microscope equipped with epifluorescence (Zeiss, Oberkochen, Germany) and photographed. C_6 cells derived from a glial rat tumor (kindly provided by Dr. G. Rebel, IRCAD, Strasbourg, France) and WCS 256 cells derived from a rat mamma carcinosarcoma (kindly provided by Dr. T. Schilling, Department of Internal Medicine I, University of Heidelberg, Germany) were used as negative controls.

Determination of cell proliferation

Renal and aortic VSMCs were studied in passages 3 through 7. Cells were seeded in 12-well plates at a density of 4×10^3 cells/cm^{-2} for determination of growth curves, population doubling time, and FBS concentration-response curves. Cell number was measured manually with a hemocytometer. Trypsinized cells of each well were counted at least three times. Cell viability was checked by dye exclusion with trypan blue, which stained generally less than 5% of the cells. For growth curves, cells were harvested from two wells by trypsinization 2, 4, 6, 8, 12, and 16 days after plating. Population doubling time was determined at several time points: between days 2 through 4 and days 2 through 6 (exponential growth phase) and between days 6 through 8. Population doubling time T was calculated as $T = (t_2 - t_1) + \ln 2/(\ln c_2 - \ln c_1)$, where $t_2 - t_1$ denotes the time difference between time points 1 and 2, and c_1 and c_2 denote cell numbers at time points 1 and 2, respectively. To study the dependence of growth on FBS concentration, cells were washed with DMEM on day 2, and medium supplemented with 0.3, 1, 3, or 10% FBS was added. The baseline value of cell number was determined on day 2 (c_1), and the effect of different FBS concentrations on cell number was measured four days later on the day 6 (c_2). Cells harvested from three wells were counted for each condition. Cell multiplication m was calculated as $m = c_2/c_1$.

Incorporation of 5-bromo-2′-deoxyuridine (BrdU) was measured as an index of DNA synthesis. For this purpose, cells were grown in 96-well microtiter plates. At confluence, cells were incubated for three days in 0.3% FBS. Cells were then exposed for 17 hours to medium supplemented with 0.3, 1, 3, or 10% FBS. After a 12.5-hour exposure to various FBS concentrations, BrdU was added for 4.5 hours at a final concentration of 10 μmol/L. BrdU incorporation was measured with a colorimetric enzyme-linked immunosorbent assay (ELISA; Boehringer-Mannheim, Meylan, France). Fixative was added for 30 minutes, and cells were incubated with a peroxidase-conjugated monoclonal mouse anti-BrdU antibody for 90 minutes at room temperature. Wells were washed three times, and the color substrate tetramethylbenzidine was added. The color reaction was stopped 10 minutes later by the addition of H_2SO_4 (0.2 mol/L final concentration). Optical density (OD) was measured at 450 nm against a reference wavelength of 690 nm with a microplate reader. Background OD, resulting from light scattering on cells and unspecific binding, was determined on cells that were incubated with BrdU for 10 minutes only and was subtracted. Measurements were done on four wells for each condition. The cell number was determined in three wells before fixation to ensure that wells contained similar numbers of renal and aortic VSMC, since the amount of BrdU incorporated depends on cell number. At least three different renal and three different aortic VSMC lines, derived from different animals, were used in each of the assays on cell proliferation.

Statistics

Data are presented as means ± SEM of the number of different cell lines used. Values measured for individual wells were averaged to obtain the mean of a single experiment. Separate experiments performed on the same cell line were averaged to calculate the mean for each cell line. To test for statistical significance, two-way analysis of variance (ANOVA), the Student-Newman-Keul's test and the nonparametric Whitney-Mann test were used as appropriate. The significance level was set to $P < 0.05$.

RESULTS

Renal vascular trees, consisting mainly of small arteries (that is, interlobar and arcuate arteries), were isolated from rat kidneys by a sieving technique first described by de León and Garcia [26]. Since the purity of renal vascular trees obtained by this method has never been tested, we measured activity of alkaline phosphatase, a tubular marker enzyme and activity of adenylyl cyclase

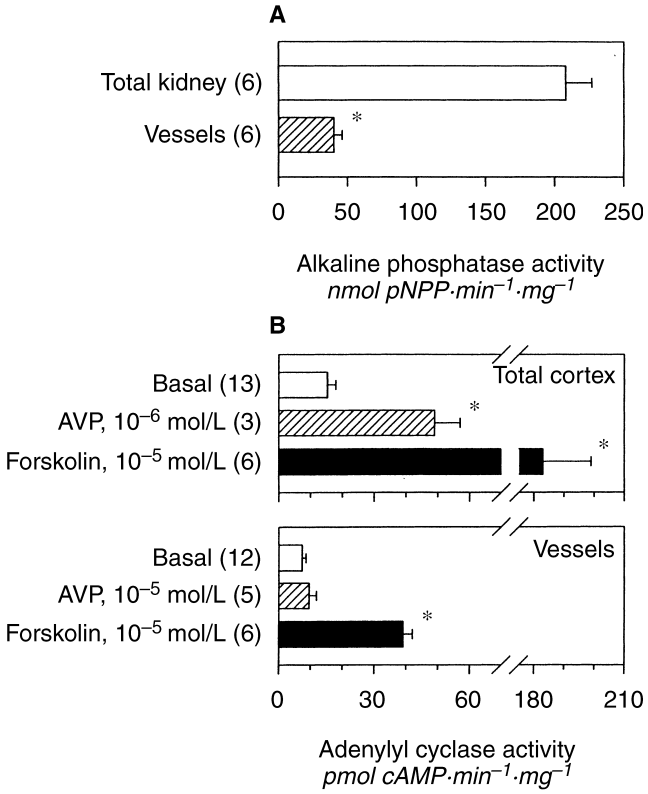

Fig. 1. Assessment of the purity of renal vascular trees. (*A*) Activity of alkaline phosphatase, a tubular marker enzyme, was decreased by 81% in isolated vascular trees as compared with total kidney homogenates. (*B*) Basal adenylyl cyclase activity was stimulated by both AVP and forskolin in membranes prepared from total renal cortex, but only by forskolin in membranes from renal vascular trees. Abbreviations are: *p*NPP, paranitrophenyl phosphate; AVP, arginine vasopressin. Data are means ± SEM for the number of experiments given in parentheses. $^*P < 0.05$ vs. total kidney or basal adenylyl cyclase activity, respectively.

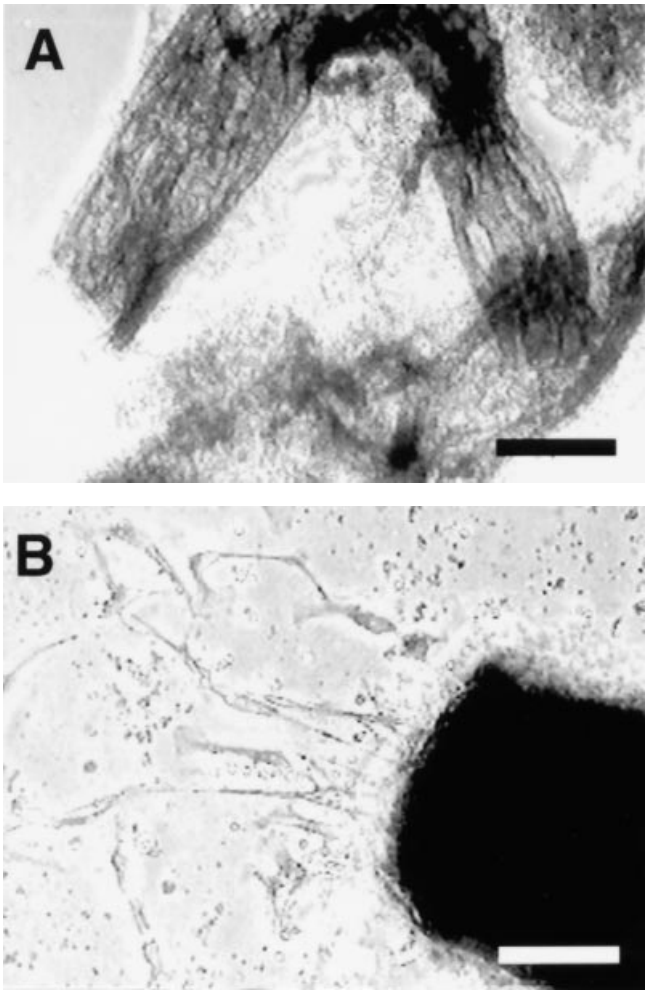

Fig. 2. Explants of renal vascular trees. (*A*) Following collagenase digestion and extensive washing, renal vascular trees were placed in culture flasks as tissue explants. (*B*) A homogenous population of spindle-shaped cells started to grew out from the explants after 5 to 10 days in culture. The cell outgrowth was frequently located at regions of open vessel cross-sections. Scale bars indicate 200 μm (A) and 100 μm (B).

in response to stimulation with AVP (Fig. 1). AVP stimulates generation of cAMP in tubules through V_2 receptors. Opposite to tubules, V_2 receptors are not found on renal vessels, which possess only V_1 receptors coupled to phospholipase C [27–29]. The activity of alkaline phosphatase was 81% lower in renal vascular tree homogenates than in homogenates of total kidney (Fig. 1A). AVP (1 μmol/L) increased andenylyl cyclase activity about threefold in renal cortical membranes (Fig. 1B). A tenfold higher concentration of AVP (10 μmol/L) had no significant effect on adenylyl cyclase activity in membranes of renal vascular trees, whereas forskolin (10 μmol/L) was able to increase adenylyl cyclase activity in both preparations.

Tubules and particularly connective tissue were further detached from renal vascular trees by collagenase treatment and extensive washing. Thereafter, renal vascular trees were placed in culture as tissue explants. Explants consisted mainly of small arteries of 80 to 400 μm in diameter (Fig. 2A). Some interlobular arteries and afferent arterioles could be detected, which disappeared within one to three days, probably because of collagenase treatment and mechanical cell damage. Occasionally, a few glomeruli were found to be still attached to the explant. However, we never observed cell outgrowth from these glomeruli, as their Bowman's capsules had remained intact. After 5 to 10 days, a uniform population of spindle-shaped cells grew out from the explants (Fig. 2B). Cell outgrowth was mainly located at regions where vessels were opened by breakage or disrupture during the isolation procedure. Sometimes we observed small cell clusters with typical epithelial or endothelial morphology at remote positions from the explants in the first days. These cell clusters, however, completely disappeared within one week. After two to three weeks, a

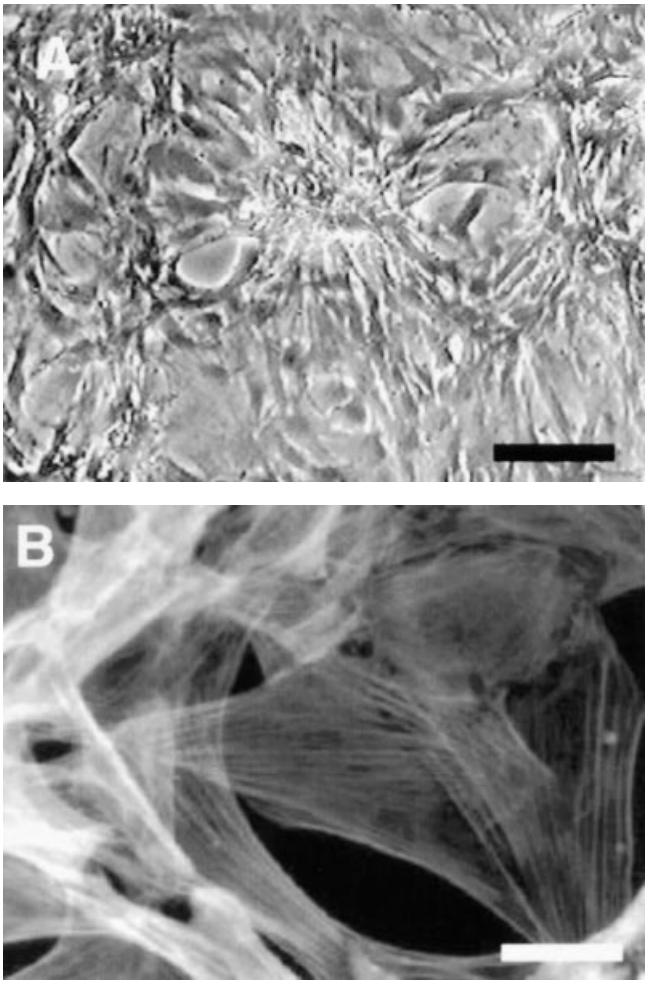

Fig. 3. Renal vascular smooth muscle cells. (*A*) Cells derived from explants of renal vascular trees grew in a hill-and-valley pattern, which is characteristic for VSMCs. (*B*) After staining with an FITC-conjugated antibody against smooth muscle α-actin, actin filaments arranged in parallel bundles were visible. Cell morphology, growth pattern, and smooth muscle α-actin expression did not noticeably change with increasing passage number. Cells in the images shown were from passages 1 (A) and 8 (B). Scale bars indicate 100 μm (A) and 20 μm (B).

sufficient number of cells had grown for subculture. As the subcultured cells reached confluence, they started to pile up at distinct locations, thus showing the typical hill-and-valley growth pattern of VSMCs (Fig. 3A). In addition, cells stained positively for smooth muscle α-actin, which was arranged in parallel bundles (Fig. 3B). Rat C_6 glial cells and rat Walker carcinosarcoma WCS 256 cells were used as negative controls and did not stain for smooth muscle α-actin (data not shown). Renal VSMCs were successfully passaged more than 20 times, without noticeable changes in morphology, growth characteristics, and smooth muscle α-actin expression.

Proliferative alterations in the renal vasculature are localized preferentially in small arteries. Therefore, we compared the growth characteristics of renal VSMCs with that of aortic VSMCs, which are the standard model for VSMC proliferation. In the presence of 10% FBS, renal VSMCs started to grow exponentially after a lag phase of two days (Fig. 4A). The growth of renal VSMCs saturated at a maximal density of $62 \pm 20 \times 10^3$ cells/cm^{-2} ($N = 5$). The population doubling time of renal VSMCs in the exponential growth phase was 36 ± 5 and 41 ± 5 hours ($N = 8$, Fig. 4B) as determined between days 2 through 4 and 2 through 6, respectively. In contrast to renal VSMCs, the growth of aortic VSMCs reached a plateau at about twofold higher cell density of $159 \pm 44 \times 10^3$ cells/cm^{-2} ($N = 4$, $P < 0.05$ vs. renal VSMCs). In addition, aortic VSMCs proliferated at a higher rate compared with renal VSMCs, with a population doubling time of 22 ± 1 and 29 ± 2 hours ($N = 5$, both $P < 0.05$ vs. renal VSMC) determined between days 2 through 4 and days 2 through 6, respectively. Following the exponential growth phase, the population doubling time (between days 6 through 8) was similar for renal and aortic VSMCs (72 ± 11 vs. 71 ± 9 h). Neither renal and aortic VSMCs used in these experiments differed significantly with respect to passage (4.6 ± 0.4 vs. 5.0 ± 0.6), nor to initial cell density on day 2 (3.4 ± 0.5 vs. $5.1 \pm 1.5 \times 10^3$ cells/cm^{-2}). In addition, population doubling time was not correlated with passage number of aortic ($r^2 = 0.07$, $P = 0.5$) or renal VSMCs ($r^2 = 0.17$, $P = 0.2$). Generally, renal VSMCs were grown from explants on collagen-coated plastic surfaces to facilitate attachment, and they were passaged in medium supplemented with 20% FBS because of their otherwise slow proliferation. Aortic VSMCs were grown from explants on uncoated surfaces, and they were passaged with 10% FBS. However, morphology and growth characteristics of both types of VSMCs did not change when aortic VSMCs were cultured according to the protocol for renal VSMCs and vice versa.

Proliferation of renal and aortic VSMCs depended on the FBS concentration employed. The concentration-response curve of FBS-stimulated cell multiplication was significantly different between renal and aortic VSMCs ($P < 0.05$ two-way ANOVA; Fig. 5A). While the cell number of renal VSMC increased $5.5- \pm 1.1$-fold from day 2 to day 6 after plating in response to 10% FBS, aortic VSMCs multiplied $10.0- \pm 1.2$-fold ($N = 5$ and 4, respectively, $P < 0.05$). Initial cell density on day 2 and passage were not significantly different between renal and aortic VSMCs (3.6 ± 0.8 vs. $5.6 \pm 1.9 \times 10^3$ cells/cm^{-2} and 4.8 ± 0.5 vs. 4.8 ± 0.8, respectively). In agreement with cell number determinations, the concentration-response curve of FBS-stimulated DNA synthesis, measured as incorporation of BrdU, differed significantly between renal and aortic VSMCs ($P < 0.05$, two-way ANOVA; Fig. 5B). In particular, renal VSMCs were quiescent at 0.3% FBS (0.016 ± 0.006 OD, $N = 3$), whereas aortic VSMCs incorporated BrdU significantly

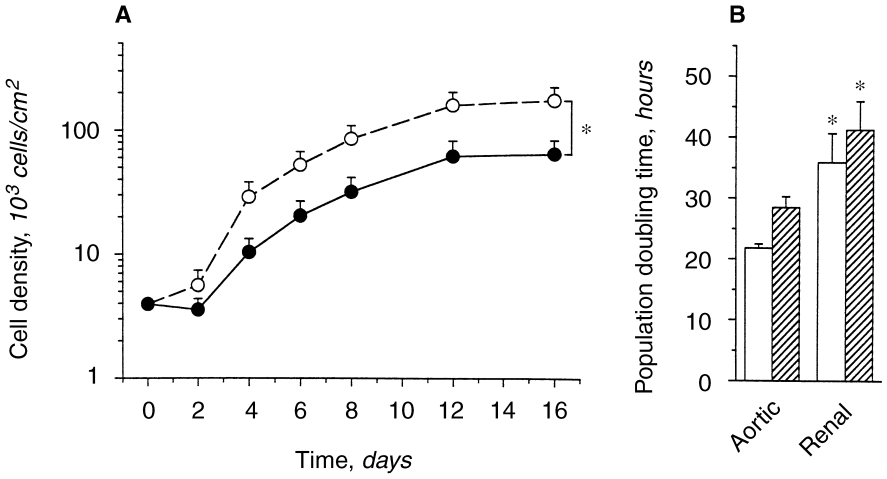

Fig. 4. Growth characteristics of renal (●) and aortic (○) VSMCs. (*A*) Growth of renal VSMCs ceased after 12 days at lower cell densities, as compared with that of aortic VSMCs in the presence of 10% FBS. Plating density (day 0) was 4×10^3 cells/cm^{-2}. (*B*) Population doubling time of renal VSMCs in the exponential growth phase was by 50% elevated as compared with aortic VSMCs. The population doubling time is shown for days 2 through 4 (□) and days 2 through 6 (▨). Data are means ± SEM. Growth curves were determined for $N = 4$ to 5 cell lines in four to five experiments; the population doubling time was measured for $N = 4$ to 8 cell lines in 4 to 13 experiments. Cells were used from passages 4 to 7. *$P < 0.05$ renal vs. aortic VSMCs.

already at this concentration (0.152 ± 0.040 OD, $N = 4$, $P < 0.05$ vs. renal VSMCs). Cell density and passage in the BrdU assays were not significantly different between renal and aortic VSMCs (19 ± 1 vs. 21 ± 5 × 10^3 cells/cm^{-2} and 3.3 ± 0.3 vs. 4.8 ± 0.8, respectively). Taken together, renal VSMCs needed about threefold higher FBS concentrations than aortic VSMCs to show similar cell multiplication and rate of DNA synthesis.

DISCUSSION

In the present study, we describe a method to culture VSMCs from small arteries of the rat kidney. To date, two methods have been presented by Dussaule et al and Dubey, Roy, and Overbeck to culture VSMC from the rabbit and rat kidney, respectively [14, 15]. In both methods, VSMCs are derived from a mixture of afferent arterioles and interlobular arteries. Larger preglomerular vessels, that is, arcuate and interlobar arteries, are excluded by virtue of the isolation technique employed. Dussaule et al isolated renal microvessels from rabbit cortex [15] by a technique developed by Helwig et al [30]. In this technique, cortical slices are passed through a tissue press, which retains small arteries because of their size and the renal vascular architecture. Microvessels are then separated from the renal parenchyma by collagenase digestion and sieving. Dubey, Roy, and Overbeck cultured renal VSMCs from explants of microvessels [14], which were isolated by magnetic separation after injection of iron oxide particles into the renal vasculature [31]. The injected iron oxide particles are trapped in preglomerular vessels. During homogenation, the iron oxide particles reside within the lumen of the arterioles, whereas they escape from large caliber vessels, which are then lost during magnetic separation.

The method for vessel isolation employed in the present study is based on a technique first described by De León and Garcia [26]. This technique has so far been used in binding studies on renal vessels [26, 32]. However, the purity of this preparation has never been checked before. Therefore, we determined alkaline phosphatase activity, which was decreased by 81% in isolated vascular trees as compared with total kidney homogenates. Zou et al reported a similar decrease of alkaline phosphatase activity by 76 and 87% in microvessels, prepared by the iron oxide technique, as compared with total cortex and tubules, respectively [20]. Helwig et al were able to decrease alkaline phosphatase activity in their microvessel preparation by 94% as compared with tubules [30]. However, the last purification step involving sucrose gradient centrifugation was omitted by Dussaule et al for VSMC culture [15], probably because of diminished cell viability. As a second index of purity, we measured adenylyl cyclase activity in response to AVP. AVP clearly stimulated adenylyl cyclase in total cortex through binding to tubular V_2 receptors, whereas it failed to do so in isolated renal vessels. V_2 receptors are not expressed in the renal vasculature of the rat, as evidenced by reverse transcriptase-polymerase chain reaction [27] and pharmacological studies [28, 29]. Thus, small renal arteries isolated in the present study can be considered to be of comparable purity as microvessels, which are obtained by the existing techniques. Because the technique of isolating small renal arteries is rather simple and rapid, it might be helpful in the molecular and biochemical analysis of the renal vasculature, supplementing existing isolation techniques.

Cultured VSMCs from renal arterioles have been used mainly to study production, receptors, and signaling pathways of vasoactive hormones [15–20]. However, deranged VSMC proliferation under pathophysiological conditions is localized preferentially in the small arteries within the renal vasculature. Prominent examples are the almost selective, early, and pressure-independent wall

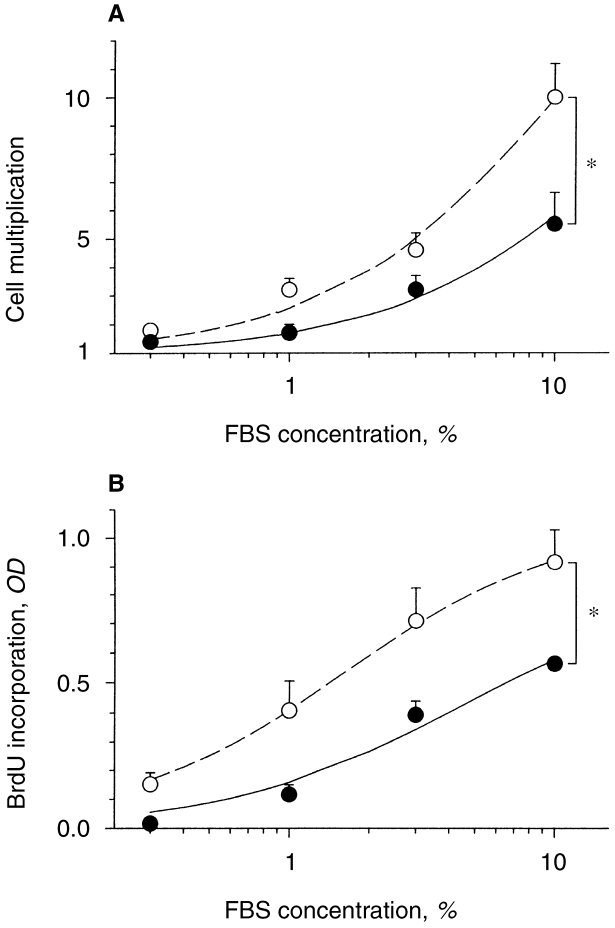

Fig. 5. Serum dependence of proliferation of renal (●) and aortic (○) VSMCs. (*A*) The concentration-response curve of cell multiplication in response to stimulation with FBS showed that renal VSMCs needed higher FBS concentrations to proliferate at the same rate as aortic VSMCs. Cell multiplication was determined as (number of cells on day 6)/(number of cells on day 2). The cell density on day 2 was 3.6 ± 0.8 and $5.6 \pm 1.9 \times 10^3$ cells/cm^{-2} for renal and aortic cells, respectively. (*B*) The concentration-response curve of DNA synthesis, measured as BrdU incorporation, in response to stimulation with FBS was also shifted to higher FBS concentrations for renal as compared with aortic VSMCs. Data are means ± SEM. Cell multiplication was determined for $N = 4$ to 5 cell lines in four to five experiments; BrdU incorporation was measured for $N = 3$ to 4 cell lines in three to four experiments. Cells were used from passages 3 through 7. $^*P < 0.05$ renal vs. aortic VSMCs.

hypertrophy of arcuate arteries in spontaneously hypertensive rats [8–10], the sudanophilic lesions in hypertension caused by nitric oxide deficiency [11], and the inflammation-triggered proliferation of VSMCs in chronic kidney transplant rejection [12, 13]. So far, mechanisms of renal VSMC proliferation have only been extrapolated from data on aortic VSMCs. However, our results demonstrate that renal VSMCs possess growth characteristics different from aortic VSMCs. Renal VSMC growth ceased at lower cell density and renal VSMCs proliferated at a lower rate in the exponential growth phase than aortic VSMCs. It could be argued that renal vessels and aortae were traumatized to a different extent during the isolation, which may result in selection of VSMCs with different growth properties. However, outgrowth of VSMCs from cultured explants started after a similar time period, indicating that VSMCs of both preparations were traumatized to a similar degree. Furthermore, dedifferentiation of VSMCs in culture might have contributed to the differences in growth characteristics between renal and aortic VSMCs. However, proliferation of VSMCs in vivo under pathophysiological conditions is likewise associated with VSMC transformation. In addition, several reports on proliferation of VSMCs, which were cultured from nonrenal vessels, support our findings on renal VSMCs. VSMCs derived from mesenteric and cerebral vessels exhibit a twofold to fourfold lower proliferation rate as compared with aortic VSMCs [33–36]. Moreover, Zhu and Arendshorst demonstrated that VSMCs cultured from preglomerular arterioles possess pharmacologically distinct angiotensin receptors as compared to cultured aortic VSMCs [19].

At present, it is unclear why growth characteristics of VSMCs of small arteries differ from those of aortic VSMCs in culture. Growth factors released by the renal parenchyma might exert profound effects on renal VSMCs, in contrast to aortic VSMCs, which lack a parenchymal environment. Our results demonstrate that higher FBS concentrations are needed to achieve a similar proliferation rate in renal VSMCs as compared with aortic VSMCs. Thus, one may conclude that either renal VSMCs generally need higher concentrations of growth factors or that growth factor requirements of renal VSMCs are different from those of aortic VSMCs, the latter being better matched by the composition of growth factors in FBS. It has been demonstrated that renal tubular cells synthesize a variety of growth factors, such as platelet-derived growth factor, insulin-like growth factor, and epidermal growth factor [37–40]. Moreover, primarily vasoactive hormones exert mitogenic effects on VSMCs through cross-talk with the mitogen-activated protein kinase pathway, like endothelin, which is released by tubular cells preferentially into the basolateral compartment [41, 42]. Thus, because of their different in vivo environment, renal VSMCs may express a different set of growth hormone and autacoid receptors as compared with aortic VSMCs.

In summary, we present a technique to isolate small arteries of the rat kidney and culture VSMCs. As compared with aortic VSMCs, VSMCs derived from small renal arteries possess distinct growth characteristics. These cells might be useful in studying deranged proliferation of VSMCs in the renal vasculature and the growth factors involved.

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